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Short-term Toxicity of Five Oils to Four Freshwater Species

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Abstract. Short-term lethality tests were conducted with five (waste oil, No. 1 fuel oil, No. 2 fuel oil, mixed blend sweet crude oil, Lloydminister crude oil) oils and four freshwater species. The oils were tested as floating layers, emulsions, and as the water-soluble fraction of 10% oil-water mixtures, in static and flow-through tests. The organisms tested were the American flagfish, *Jordanella floridae*, the fathead minnow, *Pimephales promelas*, larvae of the wood frog, *Rana sylvatica*, and larvae of the spotted salamander, *Ambystoma maculatum*. LC50 values were quite variable depending on a number of influencing factors, including the species tested, the type of oil, differences between batches of the same oil, the form of the oil when added to the test system, the type of test, duration of exposure, and the oil-water contact time.

The spillage of oil in the freshwater environment can involve significant volumes; oil spills into freshwater have been reported to reach 2.65×10^6 L quarterly (DeWitt and Melvin 1974) and as much as 3.03×10^7 L yearly (Council on Environmental Quality 1972). An additional source is the disposal of waste oil. Estimates of the waste oil input to the environment have ranged from 2.27 to 5.03×10^9 L yearly (Bernard 1971; Ostrander and Kleinert 1973; Anonymous 1974; U.S. Environmental Protection Agency 1974). Most of these oils were released into flowing bodies of water where mixing and emulsification are frequent. In spite of the volume and variety of oil released into the freshwater environment, freshwater toxicological studies with oil are relatively few (Tagatz 1961; Hokanson and Smith 1971; Hutchinson *et al.* 1972; Lappenbusch and Ward 1973; Rice 1973; Kauss *et al.* 1973; Rice *et al.* 1975; Hellebust *et al.* 1975). Much of the literature deals with cold water studies relating to northern oil exploration and transport. Since the release of oil into the environment frequently involves a sudden exposure often of a short duration, the purpose of this study was to determine the short-term lethal concentrations of oils for several freshwater species. Several factors that may influence the toxicity of oils were investigated, such as the type of oil, the form of the oil in water, and the oil-water contact time. Since much aquatic toxicological data is generated in static tests, both static and flow-through tests were conducted when possible for comparative purposes.

Materials and Methods

Lake Superior water was used as the dilution water in all tests. All flow-through tests were conducted with a modified 2-L proportional diluter (Mount and Brungs 1967; Benoit and Puglisi 1973). Temperatures in flow-through tests were maintained by mixing appropriate amounts of heated and ambient Lake Superior water in a head box prior to delivery to the diluter. The head box was vigorously aerated to maintain dissolved oxygen levels at 90 to 100% saturation previous to testing. In static tests, temperatures were maintained by placing all tanks in a constant temperature water bath.

Short-term Mortality Tests

Eight-day mortality tests were conducted with two species of fish, the American flagfish (*Jordanella floridae*) and the fathead minnow (*Pimephales promelas*). The flagfish is used as a test organism in freshwater toxicology due to its ease in culturing and the short duration of its life-cycle. The fathead minnow is probably the most commonly tested warmwater species in aquatic toxicology. Both species were obtained from laboratory cultures. Flagfish were fed live nauplii and frozen adult brine shrimp, and fathead minnows were fed Oregon moist trout starter and #3 granule PR-9 Glencoe trout food[2]. A combination of Gro-Lux and Duro-Test fluorescent bulbs was used to provide a constant

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16-hr light, 8-hr dark photoperiod at 40 ± 5 lumens at the water surface of culturing and holding tanks. At the time of testing, flagfish ranged in age from 30 to 40 days, in weight from 0.13 to 0.14 g and in standard length from 1.6 to 1.7 cm. Fathead minnows ranged in age from 30 to 60 days, in weight from 0.03 to 0.04 g and in standard length from 1.3 to 1.6 cm. The temperature for hatching, rearing, and testing of both species was 24 ± 1°C.

Few toxicological studies have used amphibians as representative aquatic vertebrates. Tests were conducted with the larval stages of the frog, *Rana sylvatica*, and the salamander, *Ambystoma maculatum*. Both species were reared from field-collected eggs. Frog larvae were fed boiled lettuce and rabbit food pellets, and salamanders were fed live brine shrimp nauplii and frog larvae. Light conditions for hatching and rearing both species was the same as for the fish. Less than 1% mortality of animals occurred in the cultures during the week before any test. At the time of testing, the ranges of age, standard length, and weight were as follows: frog larvae, 30 to 60 days, 1.1 to 1.3 cm, and 0.25 to 0.44 g; salamander larvae, 60 to 80 days, 3.9 to 4.0 cm, and 0.43 to 0.44 g. The hatching, rearing, and testing temperature for both species was 18 ± 1°C. A 0.5 dilution factor was used in all tests with duplicates for each concentration. The 25-L glass test containers were randomly arranged and in the flow-through tests received approximately ten tank volumes of toxicant solution per day. Light conditions for testing were the same as for hatching and rearing animals. Feeding was stopped 48 hr before beginning a test and was discontinued for its duration. Ten organisms were placed in each container after the addition of oil in exposures with emulsified oil and the water-soluble fraction of the oil and before the oil in the floating-oil tests. In static tests, loadings were less than 0.3 g/L. In most tests, mortalities were measured at 0, 1, 3, 6, 12, and 24 hr and daily thereafter. Death was determined by lack of movement upon gentle prodding, and dead organisms were removed when found. The LC50's, calculated by the moving average method (Harris 1959; Finney 1971), were determined only in those tests where the mortality was 65% or higher at one concentration and 35% or lower at another. A test was considered invalid if more than 10% of the control organisms died during the test. Dissolved oxygen and pH were measured at the beginning, middle, and end of each test; alkalinity and hardness were measured at the beginning and end (American Public Health Association 1971). The addition of oils caused a slight lowering of pH and dissolved oxygen concentration, but had no effect on hardness or alkalinity. The pH in all tests was between 7.1 and 7.7. The dissolved oxygen concentration was between 60% and 100% saturation in all acceptable tests. In some floating oil tests, dissolved oxygen levels dropped below 60% after eight days. Eight-day LC50's were not calculated in these cases. To prevent the driving off of volatile fractions, none of the tests were aerated. However, the methods, indicated below, used for preparation and delivery of test solutions may have resulted in the release of volatile components. Hardness and alkalinity for all tests ranged from 42 to 49 and 40 to 44 mg/L as $CaCO_3$, respectively.

Toxicant Preparation and Measurement

The oils were a waste oil, No. 1 fuel oil, No. 2 fuel oil, a mixed blend sweet crude oil, and a Lloydminister crude oil, and represent a wide spectrum of oils that may spill into the environment. Because the composition of refined products can vary from batch to batch, samples of No. 2 fuel oil were taken from two separate batches. The fuel and crude oils were obtained from Murphy Oil Corporation, Superior, WI. The waste oil was used crankcase oil obtained from the Arrowhead Refinery Company of Duluth, MN; chemical characteristics are described by Hedtke and Puglisi (1980).

Emulsified oils were prepared by blending oil and water at the required ratios in a Waring blender for two min. In static tests, the toxicant solution was prepared at the beginning of the test. For flow-through tests, fresh oil was blended with dilution water at each dilution cycle to form a new stock solution each time. This was subsequently dosed into the diluter on the following cycle. The levels of emulsified oils in the flow-through tests were monitored by fluorescence with an Aminco-Bowman spectrophotofluorimeter. For each test, at least two sets of samples were collected from each concentration plus a duplicate from one test container or from a duplicate concentration. Samples for Nos. 1 and 2 fuel oil were prepared by adding one ml of 2-propanol to each two ml of sample and measuring response at an emission wavelength of 350 mμ when excited at 234 mμ. Samples of the mixed blend sweet crude oil were extracted 1:1 with methylene chloride and, after the aqueous phase was removed, were dried with solvent-washed anhydrous sodium sulfate. The fluorescence of the extract was measured at a peak emission of 390 mμ when excited at 300 mμ. The sample preparation and analysis for emulsified used crankcase oil was the same as for crude oil, except that maximum emission was observed at 360 mμ. Two methods were used to prepare water-soluble fractions (wsf). For used crankcase oil, the solution was created by recirculating water through an oil layer (10% oil−90% water) for 18 hr and allowing the resultant mixture to separate for 6 hr. A new stock solution was prepared daily for the flow-through tests. For the other oils, solutions were prepared by repeatedly shaking 10% oil in water in a 2-L separatory funnel and allowing the mixture to separate between each shaking and before testing. The percent dilutions of the used crankcase oil wsf in flow-through tests were monitored by measuring lead with flameless atomic absorption spectrophotometry. Lead is a major contaminant of used crankcase oil because of the organometallic additivies in lubricating oil as well as that picked up during engine wear (Supp *et al.* 1973; U.S. Environmental Protection Agency 1974). By measuring lead in the test chambers and the stock solution, it was possible to determine the % dilution of the wsf present in the test chambers. Twice weekly, samples from each test container and stock solution were collected in polyethylene bottles from mid-depth and preserved with concentrated perchloric acid. At least once a week, duplicate samples were collected from a randomly selected container. In floating-oil tests, the oil was gently poured onto the water's surface with no additional mixing. Only static tests were conducted with floating oil. To determine whether the rate of response to the toxicant was dependent on oil-water contact time, in two floating-oil tests replicate test containers at the high concentrations of No. 2 fuel oil and used crankcase oil were set up with no organisms. After 96 hr, fish were placed in the tanks without coming in contact with the oil surface layer. Deaths were then recorded as in a normal test. For all oil-water systems, recovery of spiked samples compared to aqueous standards was 90% or greater, replicate samples from the same test container agreed 95% or greater, and duplicate test containers agreed 90% or greater. Since oil concentrations in static tests were unmeasured, concentrations for LC50 calculations were determined from the amount added on a volume-to-volume basis. In one static floating-crankcase-oil test, lead measurements were made after five days according to the previously described procedures. The amount of lead in the aqueous phase increased logarithmically with the amount of added oil.

Results

Calculated 96-hr LC50 values ranged from 4.9 μL/L emulsified No. 2 fuel oil for *Rana sylvatica* larvae in a flow-through test to 413,000 μL/L (41.3%) No. 2 fuel oil wsf for *Rana sylvatica* in a static test. There were several cases where there was insufficient mortality at the highest tested concentration to calculate a LC50. Table 1 includes these tests with LC50 values listed as greater than the highest tested concentration for comparison purposes. Also, for comparison purposes, most values in Table 1 are reported in μL toxicant solution/L water (ppm). It should be noted that 10000 μL/L is a 1% solution. Values for most tests with floating oils and wsf's should be appropriately considered in terms of % toxicant solution. In testing the influence of oil-water contact time on floating oil toxicity, adding organisms at the same time as the oil resulted in deaths only after 96 hr of exposure to No. 2 fuel oil and 72 hr of exposure to used crankcase oil. However, introducing organisms after 96 hr of oil-water contact time resulted in 100% mortality after 24 hr of exposure to No. 2 fuel oil and 70% mortality after 48 hr of exposure to used crankcase oil.

Discussion

The results of the short-term mortality tests indicate wide differences in the toxicity of oils. A number of factors may influence this variability: the type of oil, differences between batches of the same oil, the method of introducing the oil into the test system, the changing nature of oils with time, and the exposure time. Previous research with marine and freshwater organisms has indicated that the more refined oils are more toxic than crude or residual oils (Tagatz 1961; Anderson *et al.* 1974; Pulich *et al.* 1974). Allen (1971) reported that crude oils and heavy bunker oils were more toxic than refined oils to the development of sea urchin eggs. Large differences have also been reported among various types of crude oils (Allen 1971; Kauss *et al.* 1973; Kuhnhold 1974; Anderson *et al.* 1974; Pulich *et al.* 1974). In the present study, substantial differences were noted in the toxicity of the various types of oil, but no clear relationship was evident between toxicity and the level of oil refinement. In addition to variations in toxicity among types of oil, differences have been found between different batches of the same type of oil (Allen 1971). In the present study, floating-oil 96-hr LC50 values for the two No. 2 fuel oil batches varied from 48,300 μL/L to greater than 160,000 μL/L. Most of the values obtained with No. 2 fuel oil were with the less toxic sample. The remaining LC50's for No. 2 fuel oil could have been substantially lower if the tests had been conducted with the more toxic sample. The variable most influential on toxicity was the method of introducing the oil. Emulsified oils were substantially more toxic than floating oils and the water-soluble fractions. This increased toxicity could be caused by increased oil in the water column, by a coating of the gills, and by a more rapid release of the soluble components due to the increased surface area for oil-water contact. The differences in toxicity between floating oils and the water-soluble fractions may have resulted from a quantitative difference in the soluble components depending on the oil and the method and extent of mixing. The amount and type of agitation has been reported to effect both the amount of oil going into solution and the toxicity of that oil (Gordon *et al.* 1973; Anderson *et al.* 1974; Rice *et al.* 1975; Sniegoski 1975; Templeton *et al.* 1975). The toxicity to frog larvae of floating mixed blend sweet crude and No. 2 fuel oils was greater than that of their water soluble fractions, this suggesting that either the water extraction procedures were not effective or that larval contact with the floating layer caused increased toxicity.

In most cases, values for 96-hr exposures were substantially lower than for 24 hr. There were only a few cases when the 8-day value was lower than the 96-hr value. Most of the toxicity occurred during the first 96 hr. In tests with floating oils, the relationship between toxicity and oil-water contact time is a function of the equilibrium time for the soluble components of the oil and water. Death occurred much more rapidly when the oil was added 96 hr before the organisms than when the oil and organisms were added at the same time. The test solutions did not reach a lethal concentration for the first several days, yet once a lethal level was reached the response of the organisms was rapid. In every case where comparisons could be made, flow-through tests were more sensitive than static. In flow-through tests, the oil was added intermittently throughout the test. Static tests are likely to be less sensitive because of changes in the chemical nature and toxicity of oil with time. Oil can be modified through biodegradation (Zobell 1969; Ahearn *et al.* 1971; Bridie and Bos 1971; Kator *et al.* 1971), photooxidation (Freegarde *et al.* 1971; Klein and Pilpel 1974), and volatilization (Kreider 1971; Smith and MacIntyre 1971; Clark *et al.* 1975). Oil modified through these processes is less toxic than fresh oil (Morrow 1973; Templeton *et al.* 1975). Thus, while the concentration of oil components in water is affected by oil-water contact time, the character of the oil itself is also changing with time.

The different temperatures which were used for fish and amphibians may have influenced the toxic-

Table 1. LC50 values for five oils and four species in static and flow-through tests

Type of oil	Condition of oil when added	Type of test	Species tested	LC 50 in μL toxicant solution/L water (Confidence interval when possible) 24 hr	96 hr	8 days
Used crankcase	Water-soluble[a] fraction	Static	*Jordanella floridae*	52,500 (46,400–59,700)	36,200 (27,700–47,700)	16,800 (12,900–21,500)
		Flow-through	*Jordanella floridae*	>45,500	9,500 (6,400–17,800)	–[b]
		Flow-through	*Pimephales promelas*	20,200 (12,800–39,500)	16,600 (11,600–24,500)	16,600 (11,600–24,500)
	Emulsion	Static	*Jordanella floridae*	—	485	—
		Flow-through	*Jordanella floridae*	>200	82.7 (64.9–110)	82.7 (64.9–110)
	Floating layer	Static	*Pimephales promelas*	>40,000	12,000 (10,200–14,000)	6,200 (5,400–7,200)
			Rana sylvatica	>20,000	1,500	[c]
No. 2 fuel, Sample A	Water-soluble fraction	Static	*Jordanella floridae*	>100%[d]	>100%	>100%
		Static	*Pimephales promelas*	>100%	>100%	>100%
		Static	*Rana sylvatica*	>100%	413,000 (342,000–561,000)	—
	Emulsion	Static	*Rana sylvatica*	>62.5	26.4 (22.1–32.2)	—
		Flow-through	*Rana sylvatica*	>70.5	4.9 (3.1–7.2)	3.4
		Flow-through	*Jordanella floridae*	>81.5	60.5 (49.3–83.7)	—
		Flow-thorugh	*Pimephales promelas*	>72.6	38.6 (33.5–45.7)	34.5 (30.0–40.1)
		Flow-through	*Ambystoma maculatum*	>86.4	>86.4	>86.4
	Floating layer	Static	*Pimephales promelas*	>160,000	>160,000	>160,000
			Rana sylvatica	>80,000	<5,000	—
No. 2 fuel oil, Sample B	Floating layer	Static	*Pimephales promelas*	>80,000	48,300 (41,000–59,800)	
No. 1 fuel	Emulsion	Flow-through	*Pimephales promelas*	201 (131–428)	56.7 (39.5–83.5)	—
		Flow-through	*Rana sylvatica*[e]	>60.6	45.8 (43.4–48.7)	23.1 (12.8–36.7)
Mixed-blend sweet crude	Water-soluble fraction	Static	*Rana sylvatica*	>250,000	>250,000	>250,000
	Emulsion	Static	*Rana sylvatica*	95.1 (86.2–107)	78.0 (65.0–96.9)	78.0 (65.0–96.9)
		Flow-through	*Rana sylvatica*[e]	56.9 (51.1–65.1)	28.2 (22.4–35.3)	25.0 (19.6–31.2)
		Flow-through	*Pimephales promelas*	12.4 (9.2–16.3)	<8.4	<8.4
	Floating layer	Static	*Rana sylvatica*	21,600 (15,500–33,100)	<2,500	
Lloydminister crude	Floating layer	Static	*Rana sylvatica*	>40,000	6,300 (5,100–7,700)	

[a] Water-soluble fraction of 10% oil-water mixture
[b] Value not determined
[c] Low dissolved oxygen
[d] No mortality in 100% solution of water-soluble fraction
[e] Mean values of age, weight, and length were 60 days, 0.44 g, and 1.3 cm, respectively. Mean values for all other larval frog tests were 30 days, 0.25 g, and 1.1 cm

ity of the oils to these organisms. As a result, direct comparisons of relative sensitivity cannot be made. However, frog larvae were found to be sensitive to exposures of oil. With the exception of mixed blend sweet crude oil, LC50 values for frog larvae were lower than those for fish. Although only one test with salamander larvae was conducted, they were less sensitive than frog larvae. There was no pattern

of species sensitivity that was consistent with all tested oils. This could be due to variability in test organisms or to the difference in mode of action due to the variation in toxic components in the various oils.

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